DOCUMENT RESUME

ED 205 371 SE 035 240

AUTHOR	Plass, Richard M.
TITLE	Creature Features: A Beginner's Guide to Student Science Research.
SPONS AGENCY	New York Academy of Sciences, N.Y.
PUB DATE	Sep 80
NOTE	26p.: Not available in hard copy due to marginal legibility of original document.
AVAILABLE FROM	Director of Student Programs, The New York Academy of Sciences, 2 East 63rd St., New York, NY 10021 (no price quoted).
EDRS PRICE	MF01 Plus Postage. PC Not Available from EDRS.
DESCRIPTORS	Animal Behavior: Biology: Elementary School Science: Elementary Secondary Education: *Laboratory Animals: *Laboratory Manuals: Science Activities: Science Education: Science Laboratories: *Science Projects: *Secondary School Science: *Student Projects: *Student Research: Zoology

ABSTRACT

This booklet was prepared for use at workshops conducted by the Junior Academy of the New York Academy of Sciences and the New York Biology Teachers Association, as well as for use in the high school classroom and laboratory as an aid to both teachers and students. Its purpose is to help the beginning science research student in selecting and maintaining organisms that are commonly used in student research laboratories. Special techniques are included for bacteria, crustaceans, insects, fungi, planaria, plants, and protozoa. Possible topics for investigation are suggested. (CS)

****
* Reproductions supplied by EDRS are the best that can be made *
* from the original document. *
****

ED205371

U.S. DEPARTMENT OF HEALTH, EDUCATION & WELFARE
NATIONAL INSTITUTE OF EDUCATION

THIS DOCUMENT HAS BEEN REPRODUCED EXACTLY AS RECEIVED FROM THE PERSON OR ORGANIZATION ORIGINATING IT. POINTS OF VIEW OR OPINIONS STATED DO NOT NECESSARILY REPRESENT OFFICIAL NATIONAL INSTITUTE OF EDUCATION POSITION OR POLICY

"PERMISSION TO REPRODUCE THIS MATERIAL HAS BEEN GRANTED BY

Richard Plass

TO THE EDUCATIONAL RESOURCES INFORMATION CENTER (ERIC)."

CREATURE FEATURES

BEST COPY AVAILABLE

A BEGINNER'S GUIDE TO STUDENT SCIENCE RESEARCH

RICHARD PLASS

SE 035 240

Published by

The Junior Academy of
The New York Academy of Sciences

The New York Academy of Sciences

Paul J. Sullivan
Director of Student Programs

Toula Spetsieris
Junior Academy President

Michael Lisanti
Junior Academy Conference Director

The New York Biology Teachers Association

Paul Jablon
President

Richard M. Plass
Conference Director

Publication

This booklet was prepared for use at workshops conducted by the Junior Academy of The New York Academy of Sciences and the New York Biology Teachers Association, organizations dedicated to the promotion of science in the schools of New York City.

It is also intended for use in the high school classroom and laboratory as an aid to both teachers and students.

The Junior Academy of Sciences is a membership organization for high school students interested in the sciences, mathematics, engineering, the applied sciences and medicine. The Academy conducts a wide variety of activities of particular interest to young people.

For further information on the Junior Academy, and for additional copies of this publication, contact:

Mr. Paul J. Sullivan
Director of Student Programs
The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021

CREATURE FEATURES

A Beginner's Guide to
Student Science Research

by

Richard M. Plass

Grover Cleveland High School
Myron Liebrader, Principal

September 1980

Published by

The Junior Academy of
The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021
(212) 838-0230

ACKNOWLEDGEMENTS

A special THANKS goes to the following students of GROVER CLEVELAND HIGH SCHOOL for their tireless efforts in developing the concept for this guide, and the copious and detailed notes they supplied from their personal experiences dealing with the organisms and techniques described:

Wayne Blair, Cathy Carlson, Mary Hettich, Robert Klumpp, Kim Leibrock, Fran Mazara, Ernest Megdanis, Lucia Nicolosi, Araxi Pasagian, Barbara Sanocki, Karen Troyd, Debra Tymus, Chi Foo Yue, Joe Zabela.

Thanks also to HARVEY GOODMAN, Assistant Principal, Biology, for his inspiration and dedication to Science Research programs. And to Fran Diamant, Laboratory Specialist, without whose help there would not be a Science Research program at GCHS.

DEDICATION

This guide is dedicated to all the science research students in the past who struggled to develop usable research techniques for all those science research students in the future. Also to the School Laboratory Specialist, who will not have to explain the same things over and over again to the research students and the research teacher.

Artwork by Author.

© Copyright 1980 by RICHARD M. PLASS

at GROVER CLEVELAND HIGH SCHOOL
2127 Himrod Street
Ridgewood, Queens, New York 11385

No part of this guide may be reproduced by any means including photocopy without permission in writing from the author.

WHY DO SCIENCE RESEARCH?

If you had a chance to test yourself, what would you do? Would you run as far as you can and then run some more? Would you buy the most difficult model to build or play the most intricate song on your instrument? After you were done, and saw, heard, or felt the final product, you would probably feel extremely pleased.

It would be the kind of 'pleased', though, that cannot be explained to anyone - sort of an inside-yourself feeling of accomplishment and achievement of a personal goal. But what have you really done? Have you grown, matured, and set new goals? Did you learn how to deal with frustration, overcome obstacles, try new techniques, check through literature for help, ask advice of experts, keep notes of new methods, enter your work in a contest, win a prize, and get respect for your efforts?

If you are the type of person that enjoys these kinds of challenges, then you are a perfect candidate for a science research program.

WHAT YOU WILL NOT DO

Unless you have an extraordinary project, are working with exotic equipment, and are under the supervision of exceptional people, you should realize that the Nobel Prize is probably out of reach! On the secondary school level you will usually not be able to vivisect vertebrates, culture animal cancer cells, observe wildlife in Africa for a year, discover the missing link, stop an earthquake, or eliminate poverty and hunger from the human race forever!

If your teachers are experienced student science research instructors, they will probably not permit you to investigate psychic phenomena, parapsychology, astrology, or telepathy; experiment with pathogenic bacteria, molds, or fungi; use known human carcinogens, mutagens, or poisons; or perform state of the art research with techniques that are presently special laboratory additives. Furthermore, you should not be permitted to perform any technique or use any organism that is in any way a potential danger to you, your school, or your community.

As you see, there will be many limitations for safety reasons. You must realize from the onset that this is a very mature activity, and not all simply fun and games. There is implicit danger in any field of endeavor, particularly student science research, where students might have only part of one year of secondary school science as a foundation for some very complicated experimentation.

WHAT YOU WILL DO

The ordinary secondary school laboratory is usually well equipped to help you investigate the effect of a number of environmental variations, chemical changes, and growth patterns on a wide assortment of organisms, large and small. Almost any household chemical, food additive, or abiotic factor in any permutation can be added or subtracted from the environment of a dozen different bacteria, molds, protists, crustacea, worms, insects, or plants. You will find variations in reproductive patterns, mutations, growth, and many times life and death itself.

You will witness binary fission, color changes, bursting cell membranes, conjugations, mitosis, ameboid movement, colony formation, root hair growth, mating fruit flies, two headed worms, size and shape abnormalities, metamorphosis, antibiosis, and other serendipities beyond your imagination.

You will learn how to make solutions; culture and feed many diverse creatures; use dozens of laboratory devices; do statistical analysis; gain access to large research libraries; write to and speak to famous research scientists; attend meetings and lectures at colleges; organize term papers; and present yourself and your project to peers and adults.

You will be the mother and father to organisms that might depend upon you and only you for their very lives. That is an awesome responsibility even though they might be very small and not very consequential in the greater scheme of things.

You will learn about yourself and your ability to do independent work out of the classroom. You will get sudden flashes of

insight, invent shortcuts, design new techniques, and most exciting of all, perhaps be the only person in the entire world to ever observe some peculiar event happening on your microscope slide. This always happens, by the way, when the lab is empty and there is no one about to share the experience with. It will be the most brilliant, self-satisfying, and mature experience that will ever happen to you. You will walk through your school proud of yourself, with a secret you cannot explain to 'ordinary' students because you are not sure they could really understand or appreciate the extraordinary concentration, dedication, and determination you have undergone.

Your student science research instructor, though, will break out into a gigantic cheshire cat grin, and simply say, "Where do we go from here?"

PURPOSE

The purpose of this guide is to help the beginning science research student in the choosing, care, and keeping of organisms that are commonly used in student research laboratories. The student will also find techniques, hints, and some starting points for investigation should the organism be chosen for experimentation.

There are many more organisms to choose from than are presented here and an endless number of environmental and chemical variables that can be tested on the organisms. The organism that you finally choose should fit the kind of scientific curiosity you have. Some organisms require more time and some organisms require microscopes or special equipment that you must learn how to use.

Whatever organism and effect you choose, make sure you read all the suggested and additional literature before you begin. It is hoped that this guide will be a good starting point for your student science research project.

TABLE OF CONTENTS

INTRODUCTION.....1

PURPOSE.....2

BACTERIA.....3
- Sterilization.....3
- Handling Bacteria.....4
- Sterile Transfer.....5
- Agar Slants.....5
- Best References.....6

CRUSTACEA.....6
- Brine Shrimp.....6
- Cyclops.....8
- Best References.....9

INSECTS.....10
- Drosophila.....10
- Culturing.....11
- Etherizing.....12
- Best References.....13

FUNGI.....13
- Neurospora.....13
- Best References.....13

PLANARIA.....14
- Feeding.....14
- Water.....15
- Best References.....15

PLANTS.....16
- Germinating.....16
- Measurements.....16
- Best References.....16

PROTOZOA.....17
- Paramecia.....17
  - Feeding.....17
  - Aeration.....17
  - Culturing.....18
  - Observation.....18
- Euglena.....19
  - Culturing.....20
  - Colorimeter.....20
- Best References.....21

BIBLIOGRAPHY.....21

BACTERIA

NUTRIENT AGAR

Agar is a hardening agent. When the proper chemicals are added to it, it becomes an excellent food in which bacteria can grow. Nutrient agar is a commercial preparation and can usually be found in the laboratory. Nutrient agar can be prepared very easily and is similar to the preparation of Jello.

Preparation

Materials: Nutrient Agar, demineralized water, triple beam balance for weighing, graduated cylinder for measuring the water, scoop to transfer the agar, Pyrex beaker, hot plate, stirring rod, sterile Petri dishes (sometimes called 'plates') to hold the agar after sterilization, weighing paper, Erlenmeyer flask, asbestos gloves, and cotton.

Hint: The proportion of agar to water is always the same and usually is written on the label of the bottle of agar you get from the lab. The proportion is 23 grams of agar to 1000 milliliters of water. A simple algebra problem will give you any other amount of water or agar you need according to how many plates you want to make. An example would be if you wanted to make only 100 ml of agar liquid, you do the following math problem:

$$\frac{23 \text{ gm}}{1000 \text{ ml}} = \frac{x \text{ gm}}{100 \text{ ml}}$$

$$\frac{23\ (100)}{1000} = x$$

$$2.3 \text{ gm} = x$$

Solving for 'x' gives 2.3 grams of agar dissolved in 100 ml of water.

Usually 1000 ml of water and 23 gm of nutrient agar makes between 8 and 12 Petri dishes so you can figure out how much you need ahead of time. Assuming that you will make 1000 ml of agar, proceed as follows:

Technique: Weigh 23 grams of nutrient agar on the triple beam balance. Measure 1000 milliliters of demineralized water into the proper size graduated cylinder. Pour the water into a Pyrex beaker on a hotplate. Add the agar powder into the water and begin to boil. Stir constantly and make sure the agar does not clump together. Continue to stir the mixture while it boils. You will know when it is done because it will turn a pale, clear yellow color and will not have the original powdery look. When the agar preparation is finished, use the asbestos gloves to pour the agar into an Erlenmeyer flask. Make sure the size flask you use will fit into the Autoclave (machine that sterilizes the agar). You may have to use two Erlenmeyer flasks, each to hold half of your total agar. Asking the lab specialist is the easiest way to find the proper size flask you will need. A wad of cotton is then pushed into the mouth of the Erlenmeyer flask to seal the contents. If you know ahead of time exactly how much agar you will need, you may do all the heating in the proper size Erlenmeyer flask. This saves a lot of time.

Sterilization

Sterilization in the field of bacteriology means the killing of all living material in or on the place where you are working. If you want to use a specific bacteria or other living things, you must first make sure that other living things are not there, too. This is done by very high temperatures, very high pressures, or a combination of both. When high pressure and high temperature are needed, a machine very similar to a pressure cooker is used. This machine is called an AUTOCLAVE. The instructions for using the Autoclave are usually printed right on the machine. Most of the time, many students need their agar sterilized the same day so the general rule is to give the labeled flasks or dishes to the lab specialist and the Autoclave will be put on once each day. You will probably find your sterile and hardened agar in the refrigerator the next day. Don't forget to say "Thank you" to the lab specialist!

Cold temperature and many soaps also can cause sterilization. Usually the temperature

in the refrigerator slows the bacterial growth only. Your sterile agar may be kept sterile for several weeks this way, but should be used as soon as possible.

MAKING PETRI DISHES

Remember that agar is a hardening agent like gelatin or Jello. It will be a liquid at high temperatures and a solid at cold temperatures. In order to make your sterile, cold, and solid agar into a pourable liquid, it must be reheated and treated very carefully or it will no longer remain sterile. This means you must use STERILE TECHNIQUES. Sterile technique simply means that you take very careful steps not to expose the agar to the air for any length of time or the bacteria from the air will contaminate the agar.

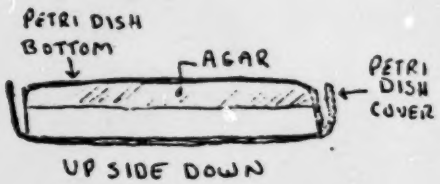
Technique: Place the Erlenmeyer Flask that contains the sterile agar in a beaker of water on the hotplate. Make sure the cotton stays in place. Heat the agar until it totally liquifies again. Since the Petri dishes are already sterile when bought, you must take care not to open them for too long. Practice moving the flask with an asbestos glove on one hand while you quickly lift the top from the petri dish with the other hand. You will probably have to do several dishes, so line them in a row. After you are more experienced, this will become a very simple technique.

Pour just enough liquid agar into the Petri dish to just cover the bottom. Cover the dish immediately! Repeat this in as many dishes as you need for your experiment. Restopper the Erlenmeyer flask with the cotton. If you have any doubts at all about the sterility of the remaining agar in the flask, ask the lab specialist to re-autoclave the flask. Return the remaining sterile agar to the refrigerator for future use.

The agar in the Petri dishes will solidify in a short while. If you see any spots that are not covered with agar, you may slowly swirl the dish until the agar spreads out fully. This must be done while the agar is still a liquid. When the agar fully solidifies, label each dish with a wax pencil and scotch tape the covers closed. The tape will remind you that the plate is still sterile, or, if you intend to use the plate right away, it will prevent you and other people from opening the dish and ruining your experiment. If you are going to use the dish right after it solidifies, tape it closed after you use it.

Hint: No matter how tightly you seal the dishes, the water from the agar will begin to evaporate and dry out the agar. A good idea is to ALWAYS store sterile or used dishes UPSIDE DOWN. This way the agar stays usable longer.

If you intend to use the dishes later, store them upside down in the refrigerator. If you placed bacteria on the agar, place the dishes in an INCUBATOR. This is a machine that is kept at 25 degrees Celsius, a temperature that is best for the growth of most bacteria.



HANDLING BACTERIA

A single bacterial cell will reproduce thousands of its own kind within a short period of time if given the ideal conditions of food, water and temperature. When grown using agar on Petri dishes, their growth is limited to the amount of food available, water in the agar, and the temperature of the incubator. The offspring cells reproduced from a single cell is called a COLONY. The colony will grow and grow until cells pile up in a way that prevents the top cells from reaching the food in the agar. Also, the bottom cells start to pollute the food with the wastes that all cells make. Eventually the colony takes on the shape and color that is typical of that species of bacteria. The colony shape and color is a helpful method of identification of the bac-

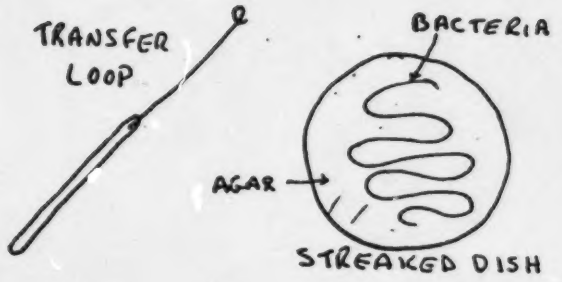
teria. When the food runs out, water is gone, pollution level is too high, or the temperature too low, the colony either completely dies or forms a type of reproductive cell called a SPORE. The spore can survive for a long time without the proper growth conditions. If you open the dish without proper care, the spores may leave and find a new place to grow. You might be that place! Used dishes must be given to the lab specialist for autoclaving when you are done with your experiment. NEVER THROW PETRI DISHES INTO THE GARBAGE! The rule is that all dishes are to be autoclaved and sterilized before they are discarded. Hopefully, you now realize why sterile technique must be used at all times in the handling and transferring of bacteria.

STERILE TRANSFER

The tool used to transfer bacteria is called by several names. Some of them are: streaking loop, innoculating needle, transfer loop, and combinations of those names. It is a piece of wire, one end of which is imbedded into a glass rod handle and the other end is curled into a loop. Sometimes the handle is metal and sometimes the wire has no loop. To INNOCULATE means to place bacteria onto a growth area using sterile technique.

Technique: The first few times you try to sterile transfer bacteria, you will wish you had three hands!

Set out your sterile agar Petri dishes and the dishes or tubes you are transferring from. You will also need a Bunsen Burner or alcohol burned to sterilize the transfer loop. Heat the tip of the wire until it glows red. This will sterilize the tip and make sure that only the bacteria you are using will go into the new dish. In a few seconds the tip will cool. Open the used dish and gently scrape the surface of the agar and remove a colony or a few colonies. Close the used dish and open the sterile dish cover. Gently rub the tip of the transfer loop on the sterile agar in a large 'S' shaped pattern. This is called STREAKING. Close and tape shut the cover of the new dish. Place your name and date on the dish taped close and place upside down in the incubator.



If you are transferring from a test tube of bacteria on agar, the technique is only slightly different. Remove the cotton from the test tube, but do not put it on the table. Play the flame of the burner on the open end of the test tube to kill any bacteria that might have been in the cotton that was there. Sterilize the transfer loop, remove the bacteria and transfer it to the new test tube or plate. Close, seal, label, and incubate the new tube or plate. All old dishes and tubes must be autoclaved before they are discarded. The area where you worked should be thoroughly washed with liquid soap obtainable from the lab specialist.

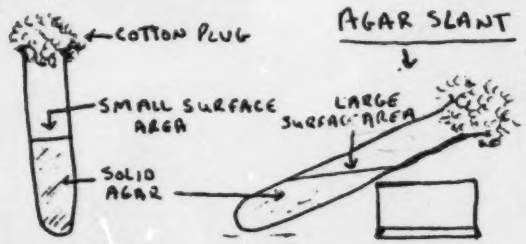
MAKING AGAR SLANTS

Sometimes you might want to store bacteria or keep a strain going and you do not want to make a Petri dish. Since the agar in any place usually dries out or the colonies cannot grow any more, students find it helpful to keep cultures going in tubes rather than dishes. These storage tubes are called STOCK CULTURES which must be transferred into fresh agar every ten days or so. You will be totally responsible for this stock culture and it will be the only source of bacteria for your research. The best way to keep this stock culture is usually in a test tube. In order to give the bacteria a large area to live and grow on the narrow surface of the agar formed by the test tube, an agar SLANT is made. The slanted agar greatly increases the surface area for bacterial growth.

Technique: Agar is prepared the usual way. You might have sterile agar already in the refrigerator that can be used. Place the flask with the solid agar in a beaker of water and heat on a hotplate until liquified. Pour a small amount of agar from the flask into the test tube. Seal the tube with cotton.

Remember that the test tube was not sterile so whether or not you used sterile agar, the test tube must be labeled and given to the lab specialist for autoclaving.

After autoclaving the cooled sterile agar in the test tubes will solidify again, so you must place the test tubes into a beaker of boiling water and reliquify the agar. When the agar is liquid again, place the test tube on an angle and wait until it re-solidifies. This will give you an AGAR SLANT. Use sterile transfer techniques to place bacteria onto the surface of the agar.



BEST REFERENCES

Breed, Robert;

Morholt (Bacteriology, p. 756), Index;

Morholt (Agar, p. 753), Index;

Oginsky, et. al;

Most microbiology textbooks.

NOTES

CRUSTACEA

Small bodies of water are sometimes subject to violent fluctuations of temperature. Small ponds, for example, may dry completely in the summer and freeze solid in the winter. Crustacea are a class of organisms in the phylum called Arthropoda that have become adapted to these rigorous conditions.

In general, adult Arthropods have jointed bodies and legs, and have an exoskeleton (external skeleton) that moves and supports the muscles and inner organs. In small crustacea, this hard exoskeleton is usually transparent, making the inner organs visible. These multicelled organisms are visible without the use of a microscope but the fine detail of their internal organs must be viewed at least under low power (100x).

The females usually produce thick shelled eggs which can resist freezing and drying. The young, called nauplii, go through several molting stages and shape changes until they reach the final adult stage. Oxygen is obtained by the use of gills, but many small crustacea can swim to the surface and make a small air bubble that they hold near their gills under water. The color of the organisms will vary as the amount of dissolved oxygen in the water changes. Small crustacea are fed a very simple diet of microorganisms that are found in the decaying egg yolk, or green algae from an aquarium.

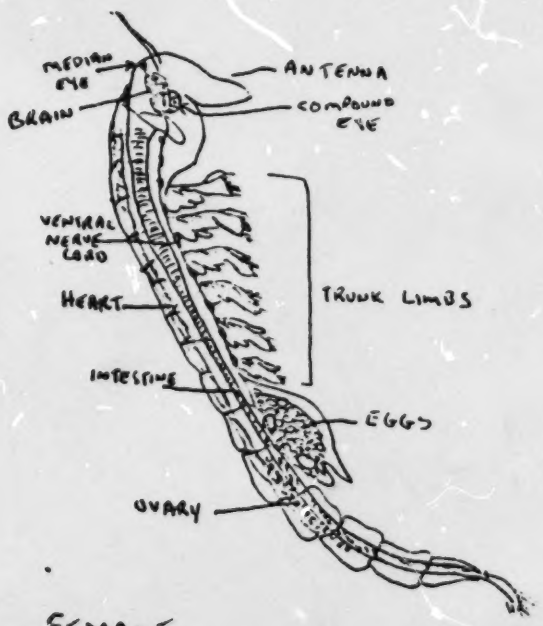
As a class, the crustaceans are a very interesting research organism. Their bodies are transparent; the young crustacea are clearly different from the adults; they can be counted and observed without a microscope; they are easily fed, they rarely need aeration; they can withstand a great variety of temperature, chemical, and other environmental changes; the males and females are easily distinguishable; the eggs last a long time; and they have a few different methods of reproduction. All of these concepts can be used to design an experimental project.

BRINE SHRIMP

Brine shrimp, otherwise known as Artemia are small crustacea found in bodies of water that have a high salt concentration. In the

United States they are found mainly along the southern California coast near San Francisco and in the state of Utah in the Great Salt Lake. The Great Salt Lake has no fresh water outlet, so the salts washed down from the hills accumulate to a concentration higher than ocean sea water. The eggs of Brine Shrimp are almost always available in aquariums or pet shops that sell fish. Live adult brine shrimp are sometimes available at these pet shops, also, since they are an excellent source of fresh protein to feed fish. The laboratory hatching of Brine Shrimp is simple and interesting to watch. Care must be taken in the salinity (salt concentration), temperature, and aeration of the water while hatching occurs.

Materials: Brine shrimp eggs obtainable from your local aquarium or pet shop; dechlorinated, demineralized, or spring water from the lab specialist; a one liter (1000 milliliter) graduated cylinder; triple beam balance; weighing paper and scoop; 55 grams of non-iodized salt (NaCl) which may be purchased as coarse Koshering salt at the supermarket; a flat glass or plastic baking pan that will hold one liter of water; medicine dropper; stirring rod; aquarium pump; airstone; and thermometer.



FEMALE BRINE SHRIMP

Technique: Brine shrimp do well in a 5.5 % salt solution. This means adding 5.5 grams of salt to each 100 ml of water. To make any larger amount, simply do an algebraic problem. For example, to make one liter (1000 ml):

$$\frac{5.5\ g}{100\ ml} = \frac{x\ g}{1000\ ml}$$

$$\frac{5.5\ (1000)}{100} = x\ g$$

$$55\ g = x$$

Solving for x = 55 grams of salt. This means that if you add 55 g of salt to 1000 ml (one liter) of water, you will be making a 5.5 % salt solution.

Measure 1000 ml of demineralized water in a graduated cylinder. Weigh 55 grams of non-iodized NaCl table salt on the triple beam balance and add it to the water. Stir until it all dissolves. One trick to learn is that many chemicals dissolve in warm water faster then cold water. Since the egg hatchings will take place faster at slightly above room temperatures (26.6 degrees Celsius or 80 degrees Fahrenheit) you might want to raise the temperature of the water before you add the salt and the eggs. This can be done by using the aquarium heater in the water or a light bulb over the pan of water. You must check the temperature for a few days to make sure it stabilizes before you add the eggs. Next weigh one gram of Brine shrimp eggs for every one liter of salt water used. Add the eggs to the 5.5 % salt water solution that has been kept at approximately 26.6° Celsius in the flat pan. At this temperature, the eggs will hatch in about one day. At lower temperatures it will take longer. Aerate constantly with an aquarium pump and airstone.

When the nauplii (baby Brine shrimp) appear, remove them with a medicine dropper and place them in a fresh 5.5 % salt water solution that is between 21° C (70° F.) and 26.6° C (80° F.). You will discover that the baby Brine shrimp and the adult stages are PHOTOTACTIC, which means they respond to and are attracted to light. This means that if you shine a light onto

one end of the pan, they will swim to that end and can easily be removed as a group with a medicine dropper.

The Brine shrimp should be fed a few grains of dried packaged yeast used for baking. The dried yeast will become living cells in the water upon which the Brine shrimp will feed. Another food may be algae (non filamentous kind) scraped off from the inside of an aquarium glass. (You might want to check the algae under a microscope to see if it grows in long filaments of attached cells or as single non-filamentous types.)

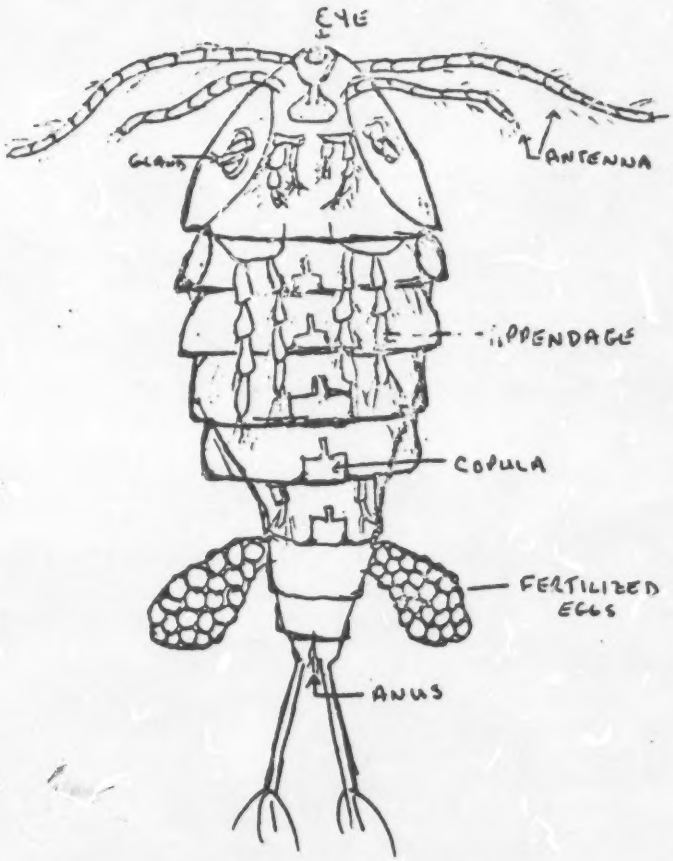
The females will develop eggs in their egg pouches in about six weeks. The Brine shrimp usually swim ventrally, belly side up, towards the light.

Research projects may be done on varying any of the hatching, growing, feeding, or reproductive conditions you used. Also, pH, colored light, trunk limb movements, chemical waste products, numbers of hatchings, swimming direction, crowding and a variety of abiotic and biotic conditions may be explored.

CYCLOPS

The Cyclops is also a crustacean but in the subclass Copepoda. Unlike the Brine shrimp, they live in fresh water; use their antennae for swimming; have no appendages in their abdominal area; and they have a single compound eye located in the center of their heads. The name Cyclops comes from the Greek story ODYSSEY by Homer, which tells of the giant, one-eyed Cyclops.

Cyclops may be found in fresh bodies of water such as local ponds but can also withstand brackish (slightly salty) water. Breeding usually takes place in warm temperatures in the summer. Females are slightly larger than the males and can be found easily since the females will have a pair of egg sacs near their rear (posterior) end. After hatching, the baby cyclops (nauplii) will undergo nearly ten moltings before they become adults. Young nauplii look very different than their adult stage. The cyclops entire life cycle may be only 30 days.



CYCLOPS

Adult cyclops are about 2 mm long and are easily seen without a microscope. Cyclops reproduce sexually, but can also reproduce by PARTHENOGENISIS. This method of reproduction does not need a male cyclops. Instead, the eggs of the female develop without fertilization.

Cyclops feed on microorganisms such as bacteria or single celled algae such as Euglena. They obtain oxygen by swimming to the surface and obtaining a bubble of air which is held near their gills, and they will do well in ordinary, dechlorinated, demineralized, or spring water without the need for aeration. Because of these factors, cyclops are an ideal research organism.

Materials: Any size culture dish; egg yolk; Euglena (alternate feeding method); dechlorinated, demineralized, or spring water; hot plate; pyrex beaker for heating; medicine dropper; culture dish cover. Students usually keep a "stock" culture of cyclops in a one gallon glass aquarium. A thriving stock culture will continuously yield dozens of cyclops for experimentation.

Technique: Hard boil an egg in the beaker of water on the hotplate. When hard boiled, cool the egg and remove the egg yolk (yellow). Add about ¼ of mashed up yolk for each gallon (about 4 liters) of demineralized water. If you use more or less water, do an algebraic problem to find the proper amount of egg yolk needed. Let the egg yolk and water stand for about five days. This will cause an excellent culture of bacteria to form. Do not aerate. Remove any film that forms on the surface of the water. If the water is very clouded, it will harm the cyclops. Remove half of the cloudy water and replace with demineralized water. Remove the cyclops from the old culture with a medicine dropper and add them to the new culture. Keep the culture container covered with a glass plate to prevent evaporation and dust from falling in. As the water near the bottom gets less and less cloudy, add very small amounts of hard boiled egg yolk. Do not remove any debris from the bottom of the culture since the eggs will fall there and hatch very soon. Sometimes a new culture will seem to totally die out and you might think of dumping the solution and starting over. DON'T DO IT! Since the life span is 30 days, the original cyclops you placed in the culture may have died but the eggs they leave behind are on the bottom of the culture ready to hatch. Wait a few days and inspect the bottom again for nauplii. This is also true for any culture in which you are doing a research project. The environmental condition you are researching might lengthen, shorten, or not affect at all their normal reproductive cycle. In any case, do not discard the culture until you are absolutely certain there are no eggs that are about to hatch. Remember that your environmental research condition might cause the time for hatching to greatly increase and you will be throwing out an excellent result!

An alternate method for feeding the cyclops is the use of the single celled plant algae called Euglena. (The care and culturing of Euglena will be found elsewhere in this booklet.) Obtain a Euglena culture from the lab specialist or from another research student and add a few drops of the green culture into the cyclops culture. Since the Euglena are green and photosynthetic, they will reproduce in the cyclops culture if you periodically place a light near the cyclops culture area.

Whichever feeding method you use, make sure the water does not become too cloudy. If it does, or you just want to freshen the water, siphon some water from near the surface, add fresh demineralized water and cover with a glass plate to keep clean.

Investigations dealing with salts, pH, waste accumulation, color due to lack of aeration, reproductive methods, sizes and shapes of nauplii, sex characteristics, feeding preference and many other biotic and abiotic conditions may be used for experimental projects.

Other crustacea that may be used in the school research laboratory that are similar in care to the brine shrimp and cyclops are the DAPHNIA which is a bit more sensitive to its environment than the others, and the OSTRACOD which has two shells that open and close around its body and is very easy to keep. The Daphnia and the Ostrocod are fresh water organisms.

BEST REFERENCES

Buchsbaum, p. 255-274;

Morholt (Artemia, p. 256), Index;

Morholt (Cyclops, p. 597-598, 42-43), Index;

Orlans, p. 94-106.

NOTES

INSECTS

DROSOPHILA

If you are interested in world hunger, genetic studies, growth and development of organisms, prefer not to use a microscope, want to do research on an organism that practically takes care of itself, and want to see results in less than thirty days for each trial, then insects are the organisms for you.

Although a few organisms can be used easily in the school research program, the insect that is most commonly used is the common fruit fly. The scientific name is Drosophila Melanogaster.

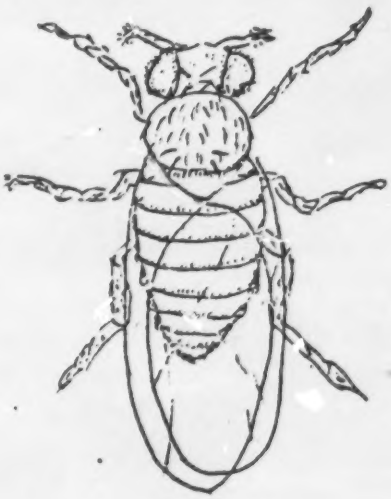
These flies are about 2 mm long (1/8") and one kind called the 'wild type' have red eyes. The males and females are slightly different in size and have body markings that are different so that students can normally tell their sex apart with only a quick look. They go through four main growth cycles that include egg, larva (worm-like stage), pupa (like a cocoon), and finally they hatch from the pupa as full grown adults. While the egg stage lasts a short time, the larvae will go through several growth stages, as the growing larva gets too big inside their skin. The larval growth stages are called INSTARS. The larva will eat their way through the food leaving thin tunnel tracks behind. They are about 3 mm long, white, and respond to a variety of stimuli. About eight or nine days after the larva hatch from the eggs and have grown to full size, they will climb up the side of the culture jar and form a pupa similar to the cocoon made by a caterpillar. Inside the pupa, the larva will undergo metamorphosis. Metamorphosis is the change of body shape, form, or function. What the larva becomes inside the pupa is a full grown adult fly. From a soft worm to a winged fly!

After a new female fly (virgin) receives sperm from a male, she will store the sperm for the rest of her life and she keeps fertilizing the eggs she produces from that moment on. In a short time she will lay 300-500 eggs and the life cycle begins again for the culture. The whole life cycle, from egg to full grown fly, is less than fifteen days. If it wasn't for the fact that you are limiting their food supply in a small culture jar, and their own wastes are poisoning their food, you would have hundreds of thousands of flies in a relatively short time.

GENETICS

Some of the most exciting and original studies on genetic mutations were done on the fruit fly. In the early part of the 20th Century, scientists exposed the fly to radiation and discovered that the offspring of these irradiated flies were sometimes unusual and mutated. This opened a whole new branch of science that dealt with genes, chromosomes, mutations, DNA, and even cancer research. It also led the scientists involved to Nobel Prizes!

MALE ♂



FEMALE ♀

FLIES FALL INTO INNER VIAL

Although the 'wild type' has red eyes and 'normal' appearance of body parts, some very unusual mutation types are well known. These can be caused to happen if the 'right' combination of chemicals and environmental conditions are present. Many of these mutated flies can be bought from commercial science supply houses and can be crossed with the wild type to see if the mutation is dominant, recessive, only found in one sex, or can be changed into another mutational type.

Some mutations include: white or some other eye color; broken, curled, or no wing at all; and large or small body size. The possible combinations of genetic crosses is almost unlimited! Even a simple temperature change will produce offspring that are larger or smaller than normal, or even have curled wings. Since eggs are usually laid before sunrise, simply changing the light/dark cycle near their culture jars will cause some change in the population size. What would happen for example, if you crossed a red-eyed female with a white-eyed male and upset their light/dark period?

Since insects are man's chief competitor for the available world food supply, it has become more and more important to fully understand their life cycles. It would be interesting to discover a natural way to disrupt their mating and reproduction without having to use poisonous insecticides that not only kill insects, but also move up the food chain and wind up in the food we eat. Many insects have mutated in recent years and no longer are affected by these poisons. Why and how they do this is included in the multi-billion dollar budget of every government throughout the world.

CULTURING

Using standard culture vials and commercially prepared food, a single pair of mated fruit flies will produce about 150 offspring before the food spoils and the entire population dies. It takes about thirty days for this whole process to occur. The moderate number of flies produced in the short time gives you time to arrange several concurrent experiments in a very small area with very little equipment and a large amount of results.

Materials: Obtain an old culture of Drosophila Melanogaster that has enough flies to choose from; a culture jar (3 mm by 100 mm or 1¼" by 4"); plastic netting that is made especially to fit inside the jar; a vial plug (spongy foam that closes the vial); a vial cover that holds the plug in place; ether (or commercially sold Fly-Nap); Drosophila food (Formula 4-24 Instant Drosophila Medium sold by Carolina Biological Supply Company or other commercial preparations); camel hair brush, anesthetizer; stirring rod; label and tape; triple beam balance; weighing paper and scoop; 10 ml graduated cylinder; and yeast.

CAUTION: The Drosophila is one of a few types of insects that respond to etherizing type chemicals. When you use ether you must use extreme CAUTION because it is very volatile (evaporates rapidly) and can be explosive if placed in warm places for too long. For these reasons you will always find the ether stored in the refrigerator and must be placed back there after you are finished experimenting. You must also work in a well ventilated area, near an open window or open door, so the fumes will not build up to a dangerous level. Take care never to work near a flame or anything that can cause a spark.

Food Culture Technique: Weigh 4 grams of Drosophila Instant Media and transfer it to the culture vial. Measure 10 ml of water and pour it into the culture vial. Mix the food and the water thoroughly. Most students experiment quite a bit in the beginning to find the ideal amount of water to use. The final food mixture should have the consistency of banana. If the food is too hard or too soft, the flies will not do well. Banana, by the way, is an excellent alternate food source to use if the Instant Media is not available. Sprinkle a very small amount of dry yeast onto the surface of the food. The yeast will cause some alcoholic fermentation which is useful to the fly and retards the growth of some unwanted organisms. If you use too much yeast, the surface of the food will harden and the flies will not be able to get food. Insert a fly net so that the flies have a place to land other than on the food. Place a label with your name, date, and some word or two to remind you what is going on in the experiment in the vial. Place a piece of clear tape over the label to hold it in place.

Close the culture vial with a foam plug and set aside.

Etherizing: In order to handle the flies for transferring to your new vial, they must be put to sleep. The anesthetizer (etherizer) has four main parts. The cap closes the ether gas area; the foam holds the ether; the inner vial is the place the sleeping flies fall into; and the cup covers the opening that the open mouth of the culture vial fits into. The concept is to let the liquid ether become a gas in the etherizing chamber, pass through the small holes in the inner vial and etherize the flies in the culture vial. After the flies from the old culture are asleep, you can sort them out by their sex, choose a suitable male and female for experimentation, and place them into your new food vial. (See page 10)

Etherizing Technique: Pour a few drops of ether onto the foam in the etherizer. Do not wet it too much. If the liquid ether touches the flies, they will die. Place the cap back on the etherizer. While you are waiting for the ether gas to fill the etherizer, take hold of the old culture and tap it onto the table. This will cause the flies to fall to the bottom of their vial and give you a chance to remove their cover and not have them escape into the room. Place the etherizer cup side up and the old culture vial mouth side down over the opening. This way the ether gas will go up into the vial and the sleeping flies will fall into the etherizer. When all the flies are etherized, pour them onto a piece of white paper. They will stay asleep for about ten to fifteen minutes. Work fast, carefully, and be well planned.

An alternate method of etherizing involves the use of a chemical commercially called FLY-NAP. This Fly-Nap is a product of Carolina Biological Supply House and may be ordered from them. It is non-explosive, does not require an etherizer, and instructions included are easy to follow It will put the flies to sleep a bit longer than ether and costs more per volume than the ether.

Choosing Flies: Females are slightly larger than males. The females have a more pointed posterior (rear) end and the male has distinct dark black markings in his posterior. Choose a male and female that appear normal for the type you are using, separate them from the other flies with a camel hair brush. (they will not stick to this type of brush), and transfer them to the new culture vial. Since the flies are still asleep, they will fall into the new food and die. To avoid this, simply place the culture vial on its side and place the flies on the plastic vial inside wall. Check back in about 45 minutes to make sure they both woke up and are free to move around. Return the original sleeping flies back to their old culture vial again making sure they do not fall into the food while they are asleep.

GENETIC CROSSES

When you are doing genetic crosses you must remember that the female fruit fly will store sperm for her entire life. If you want to be sure that the male you are using is indeed the partner that supplied the sperm, you must use a female that has not been inseminated yet. A female fly that has not yet received sperm is called a virgin. Another problem is that the female will be ready for mating six to eight hours after emerging from her pupa. This means you have to catch a female before this time to be assured of the accuracy of the genetic cross. This is really easier than it sounds since the flies almost always emerge in the morning. If you arrive in school early, etherize all the flies in the culture vial and discard them all. Close the vial and check back periodically during the day. Any female fly that emerges from the pupa during the six or seven hour school day should still be a virgin. Virgin females are recognized by their long, thin, white posterior and abdomen. When they are inseminated, they turn a bit darker and heavier looking. Etherize and transfer any virgin females you need to your new culture vial. Any male of the genetic quality you are crossing will fertilize the virgin female. Etherize and place this male into the culture. Remember to check back in a while to make sure they both survived the transfer. Within a few days, larva will appear eating through the food.

OTHER INSECTS

There is a wide variety of insects that can be used in the school laboratory. These

include the Tenebrio Molitor commonly called the meal worm that is sometimes fed to reptiles in the lab. It also goes through the egg, larva, pupa, and adult stages. They will eat ordinary dry oatmeal and can be kept in baby food jars. They molt their skin in the larval and adult stage and respond to a host of environmental and chemical stimuli. Ants, cockroaches, crickets, ladybugs, mantis, moths, and house flies can also be used.

BEST REFERENCES

Buchsbaum, p. 275-298;

Flagg, Raymond O.;

Morholt (Drosophila, p. 766), Index;

Morholt (Insects, p. 774), Index;

Orlans, p. 123-161.

NOTES

FUNGI

The pink mold Neurospora is able to synthesize its own food if fed a very simple diet called a "minimal media". From this minimal media, they can make all their essential amino acids and other organic chemicals. If the mold undergoes a mutation due to ultraviolet light or X-rays, it will not be able to synthesize the specific food chemical that minimal media chemical helped to make.

If the neurospora will not grow on the minimal media, it is evidence that the mold has undergone a mutation. The researcher simply adds small amounts of amino acids or vitamins to the minimal media until the mutated mold begins to grow. This is a very simple technique to find what mutation occurred. Since the mold will reproduce sexually, it leads the researcher into very many possible crosses to see in what ways the mutation is inheritable.

This research led to the concept that one gene controls the formation of one enzyme. An enzyme is a chemical that helps finish other chemical reactions. Performed in 1941, by the scientists Beadle and Tatum, this research won a Nobel Prize!

Hints and Techniques: Minimal media is made with ten different chemicals and water. The amounts of each and how to mix and sterilize them is explained in detail in the references below.

Neurospora crassa may be purchased as plus or minus types. These types will cross genetically with each other if placed on opposite sides of a sterile Petri dish containing minimal media. A stock culture of neurospora may be kept on a nutrient agar slant or in a Petri dish containing nutrient agar. The making of nutrient agar and the sterile transfer of the mold is the same as for bacteria and the reader is referred to the BACTERIA section of this guide. There is also an agar called Bacto-Neurospora Culture Agar made by Difco, which can be purchased from supply houses.

BEST REFERENCES

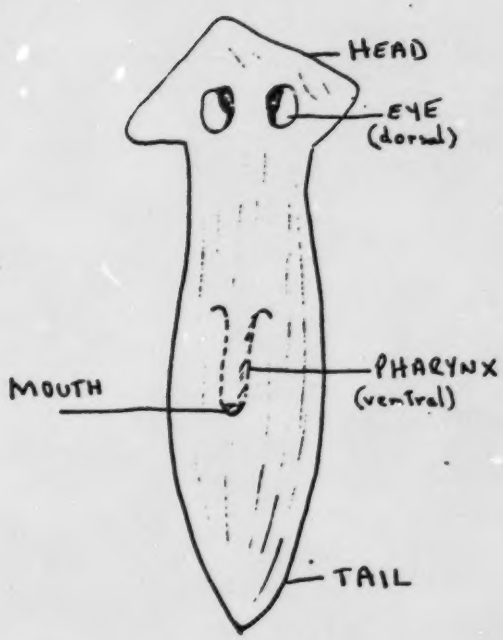
Morholt, p. 770-771, Index; (Fungi)

Morholt, p. 780, Index; (Neurospora)

NOTES

PLANARIA

Planaria are small flat worms. They are usually under 1 cm in length and live in fresh water. The species (Dugesia) commonly used in the school laboratory has a triangular, pointed head with two eyes. The eyes will appear to be "crossed" because of the dark pigmentation spot on the inside corners of a larger white eye area. They also have a nervous system that rapidly reacts to environmental stimuli such as light and they seem to prefer darker colors to lighter ones. The Planaria are carnivores and will eat meat in their water. This meat can be live, small crustacea or pieces of liver. They belong in the phylum Platyhelminthes (Greek for flat worms) which also includes parasitic worms such as the tapeworm which can cause human disease.



PLANARIAN

Planaria have several methods of reproduction which include REGENERATION. This means that they can grow new body parts if its body is cleanly cut through some section. The cut off tail can grow a new head and the cut off head can grow a new tail. Cutting them partially through the head or tail area can cause the production of a planaria with two heads and two tails. We are not sure if the two heads get along! They can also FRAGMENT or drop off a piece of their tails when they are kept in water that is not changed frequently enough. Planarians are also HERMAPHRODITIC which means they each contain both male and female reproductive systems. They will undergo SEXUAL REPRODUCTION where each planaria gives sperm and receives sperm from other planaria. Egg sacs called "cocoons" are formed which are usually 1 mm in diameter. Since this rarely happens in the school laboratory, it would be interesting to find out why.

Since the Planaria are fresh water carnivores that live under water and respond to changes in their environment they require special care of feeding, water changing and aeration.

Feeding Technique: These flatworms should do well if fed a diet of uncooked beef liver once or twice each week. Purchase beef liver from the supermarket. Cut the liver into very small pieces, about 1 cm long, $\frac{1}{2}$ cm wide, and $\frac{1}{2}$ cm deep. Wrap these small pieces in freezer wrap or foil and freeze. The purpose of cutting the liver before you freeze it is that you will only have to defrost one small feeding portion at a time.

When you are ready to feed the culture, defrost a piece of liver for fifteen minutes. A piece that is about 1 cm long, $\frac{1}{2}$ cm wide, and $\frac{1}{2}$ cm deep should be enough to feed ten planaria. Total feeding time is usually one to one and one half hours. Since their mouths are located in the middle portion of their bodies, they will climb onto the food and stay there for awhile. When they are finished, they will climb off the food and swim away. Normally their bodies are translucent but after feeding they will turn the color of the food they ingest. You may also try to feed them newly hatched Brine shrimp or fruit flies. Juices from the food will contaminate the water so the water must be

changed within two hours after feeding.

Water Changing Technique: Planaria are extremely sensitive to the type of water they are put into. The water must be almost totally demineralized. DEER PARK SPRING WATER can be purchased in almost any supermarket and has been found to be the best water to use with this organism. It has also been discovered that the planaria secrete a chemical (mucus) from their bodies that they must have in the water in order to survive. For this reason, the water must not be totally discarded when changed. Instead, pour ¾ of their dirty water into a very clean container and transfer the planaria temporarily into this container. Scrub their culture dish with a paper towel and spring water. Never use soaps of any kind. When clean, fill half way with Deer Park Spring Water and add the planaria and their dirty water that was saved. Aerate the water 15 to 30 minutes each day. Some students aerate the clean spring water before adding the planaria. Remember to aerate a bit longer before weekends.

Hints: If you do not aerate enough the planaria will line up along side the edge of the culture dish near the surface. If you do not feed them enough they will get smaller and smaller as they use up their own bodies for food. If the water becomes too foul, they will fragment and drop off their tails as a reproductive method. It is very easy to tell if they are sick. They will become much lighter in color, move sluggishly, if at all, and finally they will begin to disintegrate. You must be prepared to take them home over vacations, since they will not survive without water changing and aeration. The water must be changed at least as often as the planaria are fed. If you feed them once each week, the water should be changed at least twice each week. Planaria do very well in the dark so a dark cover should be placed on top and around the sides of their culture dish. If they are exposed to strong sunlight, the water temperatures will rise beyond the normal 20° C (68° F.) that they need for survival. Avoid radiators and unshaded window areas. In order to transfer them from container to container, touch them gently with a wooden splint. They will attach themselves to the splint and will come off if gently swished into the water in their new container.

Since they are very sensitive to chemicals in their water, experiments with pollutants should be avoided. They do respond to temperature changes, colors, light, food chemicals, and they can be trained to avoid certain stimuli. They will also regenerate at different rates depending on the factors listed above. They will also respond to how deep their water is and if the water is still or moving. The references listed below contain many varied and interesting experiments that can be done with these small flat worms.

BEST REFERENCES

Buchsbaum, p. 109-150;

Hainsworth, M. D., p. 33-46;

McConnell, J. V.;

Morholt, p. 783, Index;

Orlans, p. 46-63.

NOTES

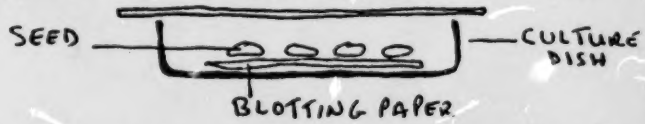
PLANTS

The green plant is the ultimate source of all food material for heterotrophs. With the advent of modern technology, also came modern pollution and very serious problems began for the survival of organisms. Put very simply, those environmental changes that affect the growth of plant life must eventually affect the growth of heterotrophs such as humans and other animals. Besides pollution problems, the world's population is rising faster than the total food output. This becomes more serious every year and leads to wars for fertile lands, famine, and starvation of hundreds of thousands of people each year. Research on the best possible growth conditions for vegetable and fruiting plants is a major concern of all world governments and is the source of billions of dollars of research funds each year. Obtaining one more gram of protein from a kilo of soybeans or rice may make the difference of life or death to the starving masses of people that depend on those crops for their existence.

As more and more of the usable land is used up, scientists have to develop methods of growing crops in arid (desert) and briny (salty) soils and at altitudes above and below normal. The kinds of fertilizers that must be used and alternate methods of water delivery must be investigated. The conditions for best seed development and the effect of chemicals in the air, soil, and water may also be researched. Natural plant growth chemicals (auxins) and natural pest control are other areas for experimentation. The simple discovery several years ago that dripping water onto the base of the stem rather than flooding the soil with water makes for a better growth rate is now being used by farmers.

Seed Growth Technique: Plant seeds are living organisms and as such need the basic requirements of all living organisms such as water and proper temperature for growth. They do not need food since they are autotrophic. Before they emerge from the soil, they have a quantity of food stored that they can draw from until they begin to undergo photosynthesis. If the seeds are planted too deep they will use up the food and die before they reach sunlight. In order to observe seed growth, germination, and development of root, stem, and leaf systems, it is adviseable to grow them without soil.

Place a piece of blotter paper on the bottom of a Petri dish or culture dish. Moisten the paper (do not flood) and place the seeds on the paper. Cover the dish with a glass plate. This is called a GERMINATION DISH.



Since the conditions are also good for bacterial and mold growth, the student may want to sterilize the seeds and the dishes before using. The best way is to mix 1 part bleach (Clorox) to 4 parts water and soak for 15 minutes. Wash the seeds with water and place on the germination dish.

Growth Measurements: Once the seeds germinate and the root is about 2.5 cm (1") long, mark the root with evenly spaced lines with India Ink. Observe the spaces between the lines after 24 then 48 hours of growth. The stem emerging from the seed may be marked in a similar fashion. A graph-like grid can be drawn on the leaves and observed after a growth interval. The pattern of the resulting grid on the leaves and the separation of the lines on the roots and stems are indications of the growth rates of these parts. The measurements can be compared to the rates for other seeds for other growth conditions.

You are urged to read as much as possible about what is known of the growth requirements of the plant you are using. Altering these conditions will provide for interesting and varied research projects. Seeds that can be used include: beans, radish, corn, vine type plants (cucumber), tomatoes, and almost any seed available at your local market.

BEST REFERENCES

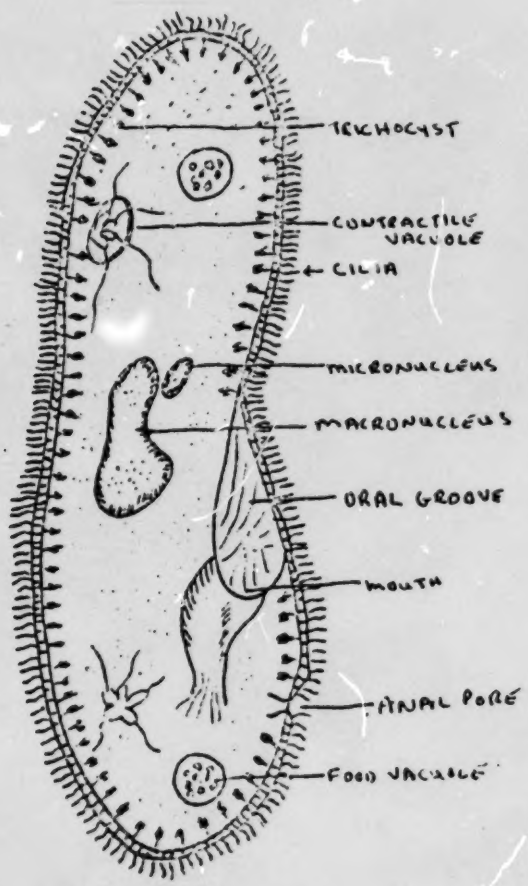
Morholt, p. 453-458.

NOTES

PROTOZOA

PARAMECIA

A paramecium is a single-celled animal which lives in ponds, streams, and fresh water bodies. As all living cells, it has certain requirements of food and temperature, and must have a fresh supply of oxygen, be able to eliminate waste chemicals, and be able to rapidly respond to changes in its environment. How it does all those things as an organism under your care creates very interesting research problems.



PARAMECIUM

Nutrition

Paramecia feed on products of decaying vegetation, bacteria, and other debris. There are several methods used to make food cultures for them. A food culture is called an INFUSION.

Materials: Large Pyrex beaker; culture dishes for keeping the paramecia; demineralized or spring water; hay; wheat grains; protozoan food pellets; medicine dropper; microscope; glass slide.

Technique: 1. Hay Infusion - Obtain a few short pieces of hay and 5 wheat grains from the lab specialist. Put the hay into about 200 ml of demineralized water in a Pyrex beaker on a hotplate and heat to boiling. When the water begins to boil, add the wheat grains. Let the system boil for a few minutes and then let cool to room temperature. Pour the cooled INFUSION into a culture dish and add the paramecia, using a medicine dropper. Always check one drop of the liquid in the medicine dropper for the presence of paramecia with a microscope. This will guarantee that you are adding paramecia to the new culture dish. Since they are microscopic, these is no other way to tell. When you add a few drops of paramecia from an old culture to a new culture it is called INNOCULATING.

2. Pellet Infusion - Obtain Protozoan Food Pellets from the lab specialist. Boil 3 pellets in 1000 ml of demineralized water. The food pellets should disintegrate. This will be enough to make 5 culture dishes. For each culture dish you plan to use, also boil 2 - 4 wheat grains. The amount of demineralized water you use in which to boil the wheat grains is not important. Divide the food pellet water into the culture dishes after the water has cooled to room temperature. Add 2 - 4 boiled wheat grains to each culture dish. Innoculate each culture dish with paramecia from the old culture. Remember to check at least one drop of the paramecia culture you are transferring with a microscope to make sure you are adding live paramecia.

Aeration

All living things need oxygen and fresh water daily. The paramecia and other living cells have the same needs as you do. How long would you live without fresh oxygen and fresh water? If you think about that for a while, you will see how dependent these organisms are on your organization and ability to care for them.

Aeration

You must aerate each culture 10-15 minutes each day. This is done by the use of an aquarium air pump attached to an airstone by plastic tube. The lab specialist will supply the pump and tube but you should purchase your own airstone since there might be an unwanted chemical left on the stone by another student's experiment. Aquarium air pumps ($3-$5), plastic tubing (10¢ per foot), and airstones (79¢) may be purchased at any pet shop. Many times local supermarkets sell these items in their pet sections.

Reculturing

Old cultures are kept alive for a long time by removing some of the surface water each day and adding fresh demineralized water. This should be done before the daily aeration. Since the paramecia are feeding at the bottom of the culture dish near the wheat grains, you will not be disturbing them. Every other day or so, you should check the culture with a microscope to make sure the culture is doing well. Remember that the paramecia are feeding nearest the food so that the sample you take should be in that area. Always take your samples before you aerate. This way you will know where they are, since the vigorous bubbling of the airstone upsets the calmness of the water. Also, you will notice that the paramecia will be moving much slower before you aerate than afterwards. The increased oxygen supply speeds up their activity. It is much easier to observe slowly moving paramecia.

The culture will go through a series of very interesting changes over a period of time. These changes are called SUCCESSIONS. When this takes place, organisms that eat paramecia will be seen in large numbers. Then in time, organisms that eat the organisms that eat paramecia will be seen. This will go on until there will be no more paramecia visible in the culture. Long before this happens you must reculture the culture. If the culture begins to smell foul, you have waited almost too long.

Reculturing is simple and should be done every 3 weeks or less. The original supply is usually all the paramecia you will get and it will be up to you to keep the future generations of cultures alive for your experimentations. The culture you draw your supply of paramecia from is called the STOCK CULTURE.

Technique: Prepare one of the two types of infusions explained before. Pour the infusions into culture dishes, aerate for a few minutes, and let cool. Add a few drops of paramecia from the old culture into the new culture. Remember to use a microscope to check to see that you are indeed adding paramecia to the new culture. Recheck in a few days to make sure the new culture is thriving and full of paramecia. You may then discard the old culture into the sink.

Hint: When you take a sample of paramecia culture to check under the microscope, always take the sample from near a food particle. Also, because the water in the medicine dropper swirls quite a bit, hold the dropper point down over the culture until the debris settles to near the open tip. Since the paramecia are attracted to gravity, they will also settle near the tip with the food debris. After you see that most of the material has gone to the tip, place a drop of this onto the glass slide on the microscope for observation.

The best temperature for keeping cultures is about 20°C to 25°C. This is near room temperature. Keep culture dishes out of direct and strong sunlight. Always keep the dishes covered with a piece of glass or paper. This keeps the temperature stable and prevents evaporation. The best pH condition is slightly basic (alkaline) at about pH 7.2. Demineralized and spring water are close enough to this pH so that no adjustments are needed.

Observation

Paramecia are microscopic and very fast moving. This makes it difficult to observe them unless you know a few very simple techniques.

Technique: Observe the paramecia before you aerate each time. You will see they move much slower before than after aeration. If you use the low power objective, look near a particle of food. They normally will

cluster around the food while feeding. If you wish to observe them under high power, you must add a chemical to their drop of water to slow them down even more. The chemical is called METHYL CELLULOSE and can be obtained from the lab specialist. Another chemical is a commercial preparation called PROTO-SLO, which will also slow them down.

Another method is to obtain glass wool from the lab specialist. This is the material that is sometimes used for filtering in fish tanks. (You must use CAUTION with this material since it is glass and will cut you.) With scissors, snip very short pieces of the glass wool over the slide. Add a drop of paramecia culture. The paramecia will become trapped between the fibers of the cut glass wool and are very easy to see especially under high power.

The paramecia can also be seen with the use of a binocular (dissecting) microscope. Although you cannot make out any details because of the low magnification, you can tell immediately if the culture is thriving.

Paramecia Types

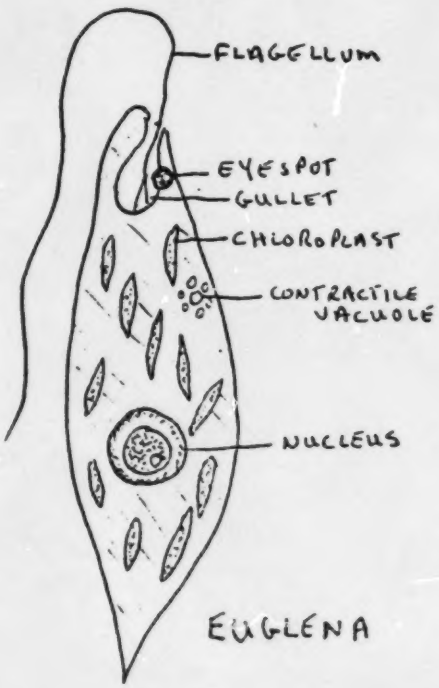
There are several different types of paramecia that are typically used in the laboratory for experimentation. Paramecia caudatum is the most common and is usually the type shown in drawings in textbooks. Paramecia aurelia is another type and is somewhat smaller than caudatum. It also has slightly different temperature and pH requirements and experimental comparisons to P. caudatum can be interesting. Another type is called the Paramecia bursaria, which is smaller than the caudatum. It is also seen as a green organism because it has a symbiotic algae plant cell called zoochlorellae living within it. How the algae cells affect the life of the Paramecia bursaria makes for interesting research.

EUGLENA

Organisms have many varied ways of gathering, using, or making food. If the organism must take in food that is already in the form of long chained molecules, it is said to be a HETEROTROPH. If the organism has the ability to manufacture its own food from chemicals in its environment, it is called an AUTOTROPH.

Protozoans such as the Paramecia are considered to be animal cells and are heterotrophic and must ingest food molecules that you supply in your cultures. Green plants and green plant cells such as the algae are autotrophs, and through the use of green chlorophyl, light energy, and chemicals in their environment and enzymes are able to manufacture their own food autotrophically in a process called PHOTOSYNTHESIS.

Somewhere in between these two types of organisms lie organisms in the order EUGLENIDA. These organisms are autotrophic in the presence of light but can also become heterotrophic when placed in darkness for a long time. They have chloroplasts and are green, and have a flagella so they can respond to and move towards light and even have a chemical 'eye' to see with. When placed in darkness, they may lose their chlorophyl and begin to ingest food as if they were heterotrophic animal cells. Certain chemicals are known to affect their ability to become green again and become autotrophic again when placed back into light areas. They will be big or small, rapid or slow moving, green or brown, form protective coverings (ENCYSTMENTS) when dried and respond unusually to antibiotics, vitamin B 12, pH conditions. All this plus the fact that they are very easy to culture and see under the microscope makes this organism a very interesting research creature.



Euglena gracilis is the usual organism used in the school research laboratory to represent the order EUGLENIDA and the class FLAGELLATA.

Materials for Culturing: To make 500 ml of culture media, gather the following materials: 500 ml of spring, demineralized, or dechlorinated water; 20 wheat grains and 15 rice grains from the lab specialist; 500 ml graduated cylinder; powdered skim or whole milk; 10 ml graduated cylinder; 500 ml Pyrex beaker; asbestos gloves; hotplate; culture dishes; scoop; medicine dropper; grease marking pencil or labels and tape.

Technique: You are going to make what is called an Euglena Medium Infusion. If you wish to make more or less than 500 ml of Infusion, adjust the amount of materials accordingly.

Measure 500 ml of demineralized water and pour it into the Pyrex beaker. Add 20 wheat grains and 15 rice grains to the water. Pour 2.5 ml of powdered milk into the small (very dry) graduated cylinder. Add the powdered milk to the contents of the Pyrex beaker. If some stick to the sides of the graduated cylinder, rinse clean with a small amount of demineralized water from the Pyrex beaker. Place the Pyrex beaker on the hotplate and bring the mixture of water, wheat, rice and powdered milk to a boil. Let boil for five minutes. Remove the Pyrex beaker from the hotplate with the asbestos gloves and let stand overnight in a cool area. Aerate the infusion using an aquarium aerator and an airstone.

Obtain a sample of Euglena gracilis from another student or the lab specialist, and check to see if the Euglena are thriving by placing a drop on the microscope slide and observing under low power. If the culture looks to be a pure Euglena, transfer a few medicine droppers full from the old culture to your new infusion. The new infusion culture should be milky white and will turn green as the Euglena reproduce in their new environment. If you are making several cultures, evenly divide your infusion into the culture dishes, label them, and transfer the Euglena from the old culture to your new ones. Adding Euglena to a new culture media is called INNOCULATING. The culture dish or large jar in which you keep your personal supply of Euglena for your research project is called the STOCK CULTURE. To use the new words properly, you would say that you are INNOCULATING Euglena from your STOCK CULTURE to your new INFUSION. Keep your cultures covered!

Hints: Since Euglena are both autotrophic and heterotrophic, any project you design should have the same number of culture dishes in the light as in the total dark so you can compare their growth rate for each nutrition type for the variable you choose to research.

The depth or intensity of green color that the Euglena appear to have is a function of the amount of chlorophyl and the number of Euglena that are present in the culture. If you look at two different culture dishes that received different amounts of your experimental condition after the same number of days, you might notice that to your eye they appear to be different shades of green. This is because the condition you used changes their growth rate or their ability to synthesize chlorophyl. A simple way of finding actual color data is to use a machine called a COLORIMETER. By passing a beam of white light through a test tube full of Euglena culture, the machine will produce a number on its meter dial that will indicate the amount of 'greenness' of the solution. This is called the PER CENT TRANSMITTANCE, where the lower the per cent reading is, the less light can go through the green solution hence indicating darker green or more growth in comparison to a solution that has a higher per cent transmittance of light showing lighter green and less growth. Comparisons must be for the same number of days from the beginning of the trial. Using the machine is very simple and any experienced student or lab specialist can teach you how to use it in 2 minutes!

Since the Euglena are flagellated and also contain an 'eyespot', they will move towards the light. This response is called a PHOTOTAXIC response. You can collect a large number of Euglena from a stock culture by covering the entire beaker with

black paper except for a small cutout 'window' on one side. You place the beaker in a way that has a light source facing the window. In a few minutes, the Euglena will move to the inside of the beaker and cover the window. You then simply remove this concentrated Euglena sample with a medicine dropper or scrape them off the side of the glass with a scalpel and transfer them to your new culture.

Euglena are aerobic and have a need for oxygen in their culture water. This means that you must aerate the water 10-15 minutes each day. If you are to be absent, inform another trustworthy student to do this for you.

You will also discover that the Euglena move more rapidly after you aerate than before. If you are going to do a microscopic examination of the Euglena, it is recommended that you do so before you aerate. You might want to refer to the Paramecia technique of adding Methyl Cellulose or PROTOSLO to the slide to slow their movements even further.

Periodic freshening of the water is also recommended to avoid the buildup of fouling wastes. To do this, simply pour or siphon off one third of the culture every few days and add more demineralized water. Reculturing should be done when the water begins to smell or microscopic examination shows the presence of other organisms that feed on the Euglena. This should be done every two weeks. A thriving, well cared for stock culture should always be available so that you will have a source of Euglena whenever you begin a new trial in your research.

BEST REFERENCES

Buchsbaum, p. 11-58;

Curtis, Helena;

Hall, Richard P.;

Kudo, R. R.;

Morholt, p. 768, 785, Index;

Orlans, p. 12-35;

Schwartz, George;

Whitten and Pendergrass.

BIBLIOGRAPHY

Breed, Robert. Bergey's Manual of Determinitive Bacteriology. Baltimore: The Williams and Wilkins Company, 1957.

Buchsbaum, Ralph. Animals without Backbones. Chicago: The University of Chicago Press, 1938.

Curtis, Helena. The Marvelous Animals - An Introduction to the Protozoa. Garden City: Natural History Press, 1966.

Flagg, Raymond. Carolina Drosophila Manual. Burlington: Carolina Biological Supply Company, 1975.

Hainsworth, M. D. Experiments in Animal Behavior. Boston: Houghton Mifflin Company, 1967.

Hall, Richard. Protozoa - The Simplest of All Animals. New York: Holt, Rinehart and Winston, 1964.

Kudo, R. R. Protozoology. Springfield: Charles C. Thomas, 1966.

McConnell, J. V. A Manual of Psychological Experimentation on Planarians. Ann Arbor: J. V. McConnell, 1967.

Morholt, Evelyn, Paul Brandwein, and Alexander Joseph. A Sourcebook for the Biological Sciences. New York: Harcourt, Brace and World, Inc., 1958.

Oginsky and Umbreit. An Introduction to Bacterial Physiology. San Francisco: W. H. Freeman and Company, 1959.

Orlans, F. Barbara. Animal Care From Protozoa to Small Mammals. Menlo Park: Addison-Wesley, 1977.

Schwartz, George I. Life in A Drop of Water. New York: Doubleday and Company, 1970.

Whitten, Richard H. and William R. Pendergrass. Care of Lower Invertebrates. Burlington: Carolina Biological Supply Company, 1977.